Evaluation of the physicochemical and biological characteristics of soils in organically managed vineyards with different establishment systems

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Abstract

Soil remains a poor characterised component of the viticulture system, especially the soil microbiota and its importance for plant-soil system. Having a complete understanding of the soil characteristics of vineyards can lead to a better understanding of the vine’s behaviour and more efficient and sustainable management of the vineyards.

The present study aimed to evaluate the functional biodiversity and physico-chemical characteristics of soils from organically managed vineyards and two scenarios: i) Vineyards from Douro region and with different establishment systems; ii) Vineyards from different regions (Lisbon and Douro) but with similar establishment system. These scenarios allowed the assessment the impact of the establishment system on soil quality and the regional variability of soil quality.

This study was conducted in two vineyards from Demarcated Region of Douro, in Quinta dos Murças, and one vineyard from Lisbon region, in Quinta do Monte D’Oiro. In Quinta dos Murças, the vineyards were established in 2010 in a narrow terrace system and a vertical planting system, while in Quinta do Monte D’Oiro the vineyard was planted using a traditional planting system in 2012. In all vineyards, the variety was Touriga Nacional and the rootstock 1103 P. Soil chemical properties such as pH in water, electrical conductivity, soil organic carbon, total N, concentration of macro- and micro-nutrients in available and total fractions, cation exchangeable capacity and acid/non-acid exchangeable cations, % fine/coarse fractions, and humidity were analysed. Biological parameters were determined by several enzymatic activities associated to overall microbial community (dehydrogenase) and nutrient cycles (Urease, Acid phosphatase, Sulfatase, Cellulase and β-glucosidase).

The results indicated that the physico-chemical soil quality in Quinta do Monte D’Oiro is adequate for vineyard culture, with high availability of most nutrients, namely Fe, Mg, N and P, and high organic matter contents. Additionally, the cation exchange capacity had a high value reflecting, indirectly, the soil fertility. On the other hand, vineyards from Douro showed low total N and organic carbon concentrations, what can explain low enzymatic activities, as well as low available concentrations of most micronutrients (e.g., Mn, Zn, Cu and K). The CEC was also low, which can be related to the low levels of organic matter in the soil. In Douro vineyards, only the CEC differed between establishment system, being superior in the Narrow terrace vineyard.

Comparing soils from the two studied regions, there were significant differences in some soil enzymatic (e.g., Acid phosphatase, Sulfatase, Cellulase) and physico-chemical parameters such as organic carbon, total N, CEC and nutrients in the available fraction (e.g., Cu, Fe, Mn, Zn and P). These parameters were significantly higher in Quinta do Monte D'Oiro, which can be associated to edaphoclimatic factors and management practices. Moreover, the study did not show significant correlations between biological and chemical parameters.

Thus, this study can be considered important for the improvement of the chemical quality of vineyard soils and stimulation of biological activity for auto-sustainability of the agricultural system. Additionally, it is essential that the evaluation continues in order to understand how chemical and biological soil quality in vineyard soils contribute to the plant performance and, in some cases, how the soil quality can be improved (e.g., through green technologies).

Keywords: Mountain Viticulture; Narrow Terrace; Vertical Planting; Enzymatic soil activities; Rhizosphere.

Avaliação das características físico-químicas e biológicas de solos de vinhas em modo de produção biológico com diferentes sistemas de instalação

Resumo

O solo continua a ser um dos componentes mais mal estudados no sistema agrícola que é a vinha. Perceber os vários parâmetros do solo poderá levar a uma melhor compreensão do comportamento da videira e por sua vez, a uma gestão mais eficiente da cultura da vinha.

O presente trabalho teve como objetivo a análise da biodiversidade funcional e das propriedades físico-químicas em vinhas em modo de produção biológico. Este estudo foi realizado em dois cenários distintos: i) Vinhas com modo de instalação distintos (patamares e vinha plantada ao alto); ii) Vinhas localizadas em regiões distintas (Lisboa e Douro). Esta análise permitiu perceber o impacto do sistema de instalação na qualidade do solo e variabilidade regional da qualidade do solo entre regiões.

Este estudo foi realizado na Região Demarcada do Douro, na Quinta dos Murças, e na Região Demarcada de Lisboa, na Quinta do Monte D´Oiro. Na Quinta dos Murças, foram analisadas duas vinhas: uma vinha instalada em patamares, característicos da região, e outra instalada na vertical. Ambas as vinhas foram instaladas em 2010. Na Quinta do Monte D´Oiro, a vinha analisada foi plantada usando o sistema de plantação convencional. A vinha na Quinta do Monte D’Oiro foi instalada em 2012. Em todas as parcelas a variedade plantada é Touriga Nacional, associada ao porta-enxerto 1103 P. As amostras de solos foram analisadas quimicamente para: pH em água, condutividade elétrica, carbono orgânico total, N total, concentração de nutrientes nas frações disponível e total, capacidade de troca catiónica e catiões ácidos/não ácidos de troca, % da fração fina/grosseira e humidade. A biodiversidade funcional do solo foi interpretada tendo por base várias atividades enzimáticas, nomeadamente a Desidrogenase, Fosfatase Ácida, Urease, Celulase, Sulfatase e β-Glucosidade.

Os resultados mostraram que a vinha na quinta do Monte D’Oiro tem um solo rico em nutrientes disponíveis, nomeadamente Fe, Mg, P e N. O solo mostrou uma elevada capacidade de troca catiónica. O solo desta vinha é também muito rico em carbono orgânico e tem uma componente biológica muito ativa. As vinhas na Quinta dos Murças apresentaram baixa disponibilidade de nutrientes (Mn, Zn, Cu e K), e solos pobres em matéria orgânica e N total. A capacidade de troca catiónica das vinhas na Quinta dos Murças, mostrou níveis baixos, o que pode estar relacionado com os baixos níveis de carbono orgânico no solo. Para além disso, as atividades enzimáticas do solo são menores do que as da vinha da região de

Lisboa. Comparando sistemas de instalação, apenas as características do complexo de troca catiónica, como a capacidade de troca catiónica e a percentagem de saturação em bases, diferiram significativamente.

Entre regiões, existem diferenças significativas em quase todas as propriedades físico-químicas, como o nível de carbono orgânico, N total, e teores de vários nutrientes na fração disponível (Cu, Fe, Mn, Zn e P). Para além disso, certas atividades enzimáticas também diferem significativamente (Sulfatase, Fosfatase Ácida e Glucosidase). Estes parâmetros eram significativamente superiores na Quinta do Monte D'Oiro, o que poderá estar associado a fatores edafoclimáticos e práticas culturais realizadas. O estudo não demonstrou correlações relevantes entre as componentes biológicas e físico-químicas dos vários solos em estudo.

Este estudo pode ser considerado importante, pois revela informação útil para a melhoria da qualidade química de solos e estimulação da atividade biológica para autossustentabilidade de sistemas agrícolas. Para além disso, em estudos posteriores seria importante perceber como a qualidade do solo e a sua biodiversidade funcional em vinhas orgânicas pode contribuir para o desempenho da planta, e até que ponto a qualidade do solo pode ser melhorada.

Palavras-chave: Viticultura de Montanha; Patamares Estreitos; Vinha ao alto; Atividades enzimáticas do solo; Rizosfera.

Resumo alargado

O conhecimento integral das características do solo associadas a uma determinada cultura é fundamental para o melhor entendimento dos comportamentos ecofisiológicos e desenvolvimento das plantas e, consequentemente, da produtividade da cultura, permitindo assim uma gestão mais eficiente e sustentável da vinha.

A indústria da vinha e do vinho é um importante sector económico em Portugal. Portugal é o 8º país com maior área de vinha do mundo (190 000 ha) e o 11º maior produtor de vinho do mundo. Nos últimos anos a exportação do vinho português para todo o mundo gerou receitas muito significativas. A produção biológica está em amplo crescimento existindo atualmente na Europa cerca de 300 000 ha.

O estudo dos parâmetros físico-químicos dos solos e a sua influência nas comunidades microbianas do solo e no comportamento das mesmas assumem particular importância já que, têm um grande impacto no desenvolvimento, produtividade da cultura e perenidade. A biodiversidade funcional do solo, a qual pode ser avaliada através de diferentes atividades enzimáticas, é considerada fundamental na regulação de vários dos processos edáficos, por exemplo dos níveis de nutrientes ou decomposição da matéria orgânica. A análise detalhada e integral de solos de vinhas no modo de produção biológico é recente. No entanto, pode ser considerada crucial para um melhor entendimento dos efeitos deste modo de produção na qualidade solo e, consequentemente, na produtividade da vinha.

O presente trabalho teve como objetivo a análise da biodiversidade funcional e das propriedades físico-químicas em vinhas em modo de produção biológico. Este estudo foi realizado em dois cenários distintos: i) Vinhas com modo de instalação diferentes (patamares e vinha plantada ao alto); ii) Vinhas localizadas em regiões distintas (Lisboa e Douro). Esta análise permitiu perceber o impacto do sistema de instalação na qualidade do solo e variabilidade regional da qualidade do solo entre regiões.

Este estudo foi realizado na Região Demarcada do Douro, na Quinta dos Murças, e na Região Demarcada de Lisboa, na Quinta do Monte D´Oiro. Na Quinta dos Murças, foram analisadas duas vinhas: uma vinha instalada em patamares, característicos da região, e outra instalada na vertical. Ambas as vinhas foram instaladas em 2010. Na Quinta do Monte D´Oiro, a vinha analisada foi plantada usando o sistema de plantação convencional. A vinha na Quinta do Monte D’Oiro foi instalada em 2012. Em todas as parcelas a variedade plantada é Touriga Nacional associada ao porta-enxerto 1103 P. As amostras de solos foram analisadas quimicamente para: pH em água, condutividade elétrica, carbono orgânico total, N total, concentração de nutrientes nas frações disponível e total, capacidade de troca catiónica e

catiões ácidos/não ácidos de troca, % da fração fina/grosseira e humidade. A biodiversidade funcional do solo foi interpretada tendo por base várias atividades enzimáticas, nomeadamente a Desidrogenase, Fosfatase Ácida, Urease, Celulase, Sulfatase e β-Glucosidade.

Através da análise de dados verificou-se que a vinha na Quinta do Monte D'Oiro apresenta níveis elevados da maior parte dos nutrientes disponíveis (ex. Fe, Mg, P e N), bem como carbono orgânico e azoto total, indicando uma significativa fertilidade. A capacidade de troca catiónica apresentou também um valor bastante alto, estando possivelmente relacionado com os níveis elevados de matéria orgânica no solo. Estes dados podem explicar as importantes atividades enzimáticas determinadas. Os solos na região do Douro, embora adequados à cultura da vinha, possuem níveis considerados baixos de carbono orgânico e azoto total, bem como uma baixa capacidade de troca catiónica. Relativamente à disponibilidade dos nutrientes, os solos de ambas as vinhas (independentemente do sistema de plantação) mostraram baixas concentrações de Mn, Zn, Cu e K. A análise da componente biológica mostrou atividades enzimáticas inferiores em relação à região de Lisboa.

Quando comparando sistemas de instalação distintos na mesma região apenas existiam diferenças significativas no complexo de troca catiónica, nomeadamente na concentração de certos catiões de troca (Ca, Mg e Ca) e consequentemente na capacidade troca catiónica (7 vezes superior no sistema de patamares estreitos) e o grau de saturação em bases. Por outro lado, não houve diferenças significativas entre atividades enzimáticas.

A grande maioria das características físico-químicas e biológicas do solo mostraram-se significativamente diferentes entre regiões. O nível de carbono orgânico, N total, e teores de vários nutrientes na fração disponível (Cu, Fe, Mn, Zn e P) são significativamente superiores na região demarcada de Lisboa. Relativamente à componente biológica do solo, várias atividades enzimáticas mostraram resultados significativamente superiores na região de Lisboa, como a Sulfatase, Fosfatase Ácida e a Glucosidase. Estas diferenças significativas podem estar associadas a fatores edafoclimáticos. Estes fatores, associados a algumas práticas de gestão da cultura distintas (nomeadamente nas práticas culturais e na presença de rega da vinha), podem conduzir às diferenças significativas observadas. Por fim, não foram encontradas correlações entre as atividades biológicas e as características físico-químicas dos solos.

Este estudo apresenta uma análise físico-química e biológica completa de vinhas em modo de produção biológico, que poderá ser usado para comparações futuras entre vinhas com modos de produção distintos. O trabalho revela informação útil para a melhoria da qualidade química de solos e estimulação da atividade biológica para autossustentabilidade de sistemas

agrícolas. Permitiu também a obtenção de informação que pode ser importante para sustentar os estudos futuros relativamente ao impacto da qualidade várias características físico-químicas e biológicas do solo, sanidade e produtividade da videira. Para além disso, futuramente seria importante perceber como a qualidade do solo poderá ser melhorada para uma promover o desempenho da cultura.

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List of Abbreviations

BS%: Base saturation percentage

CEC: Cation exchange capacity

CEL: Cellulase

DDR: Demarcated Douro Region

DHA: Dehydrogenase

EC: Electrical conductivity

GLU: β-glucosidase

OIV: International Organisation of Vine and Wine

PHO: Acid phosphatase

SOC: Soil organic carbon

SUL: Sulfatase

URE: Urease

VSP: Vertical shoot positioning

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1. Introduction

The treatments made in a vineyard, from chemical to biological, play a relevant role in the vineyard sustainability. The main goal of such practices is to maximize crop species' health, resilience, and productivity. Nowadays, the public is more aware of the impact of agrochemicals. Consequently, alternative vineyard management philosophies have emerged, like organic management. Worldwide, the organic vineyard management has increased in the last years, and there are some studies about the impact of organic management on vines (Döring et al., 2015, 2019; Rienth & Scholasch, 2019; Tassoni et al., 2013). However, the evaluation of soil associated with organic management still is limited (Liu & Howell, 2020). Nonetheless, understanding the impact of properties of the soil on the vineyard aim at helping producers optimize the way organic vineyards are managed, positively affecting their sanity and productivity. Considering the importance of soil quality on the performance of culture, understanding how some management practices affect the soil, and consequently the vines, would also be important.

When installing a vineyard, many decisions have to be made. One crucial decision regards what establishment system to use when planting vines, as it will restrain many vineyard characteristics (e.g., topography of the plot, density of plantation, training system). Thus, by studying vineyards with different establishment systems, it will be possible to see the consequences of an establishment system on the properties and quality of the soil, and consequently, the vine itself.

1.1. Objectives

The main goal of this work was to evaluate the physico-chemical properties and functional biodiversity of soils from organically managed vineyards in two scenarios: i) Vineyards from Douro region and with different establishment systems; ii) Vineyards from different regions (Lisbon and Douro) but with similar establishment system. These scenarios allowed the assessment the impact of the establishment system on soil quality and the regional variability of soil quality.

The existence of correlations between physico-chemical and biological properties of the soils was also tested.

All the vineyards in the study presented the same varieity (Touriga Nacional) and rootstock (1103 P). Moreover, the vineyards in Douro were planted in 2010 and the vineyard in Lisbon was planted in 2012.

2. Literature Review

2.1. Organic Agriculture

To better understand the actions taken in organic management, it is vital to understand this kind of practice's objective and principles. According to European Union Regulation (2018), organic production is an overall system of farm management and food production that combines best environmental and climate action practices, a high level of biodiversity and the preservation of natural resources.

Having this in mind, the objectives of Organic Production, according to the Regulation (EU) 2018/848, are to:

a) Contribute to the protection of the environment and the climate;
b) Maintain the long-term fertility of soils;
c) Contribute to a high level of biodiversity;
d) Contribute to a non-toxic environment;
e) Contribute to high animal welfare standards and to meeting the species-specific behavioural needs of animals;
f) Encouraging short distribution channels and local production in the various areas of the Union;
g) Encourage the preservation of rare and native breeds in danger of extinction;
h) Contribute to the development of the supply of plant genetic material adapted to the specific needs and objectives of organic agriculture;
i) Contribute to a high level of biodiversity by using diverse plant genetic material, such as heterogeneous organic material and organic varieties suitable for organic production;
j) Foster the development of organic plant breeding activities to contribute to the organic sector's favourable economic perspectives.

2.2. Organic Viticulture

The most widespread system in viticulture is the integrated cultivation. Nonetheless, over the past 15 years, there has been an increasing trend towards organic viticulture (Hendgen et al., 2020).

In fact, International Organisation of Vine and Wine (2021) reported that the organically managed vineyard area in the world has increased significantly since 2004 (Figure 1). Also, Europe has about 90 % of the world’s total area of organic vineyard, 500 000 ha. The countries with the largest area of organically managed vineyards are Spain, Italy, and France (OIV, 2021).

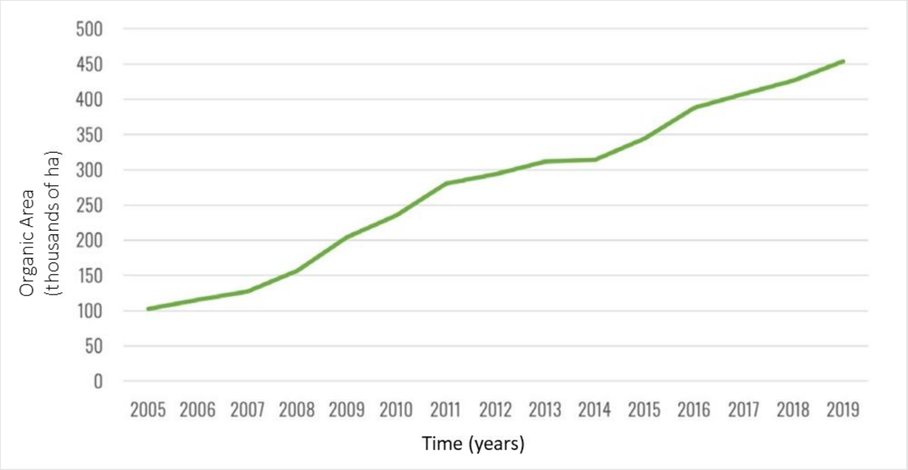

Figure 1- Development of the organic vineyard area in the world between 2005 and 2019 (OIV, 2021).

In Portugal, the same tendency has been observed. The organic vineyard certificated area is increasing ≈184 ha every year, reaching 4000 ha in 2019 (Figure 2; FILB, 2019).

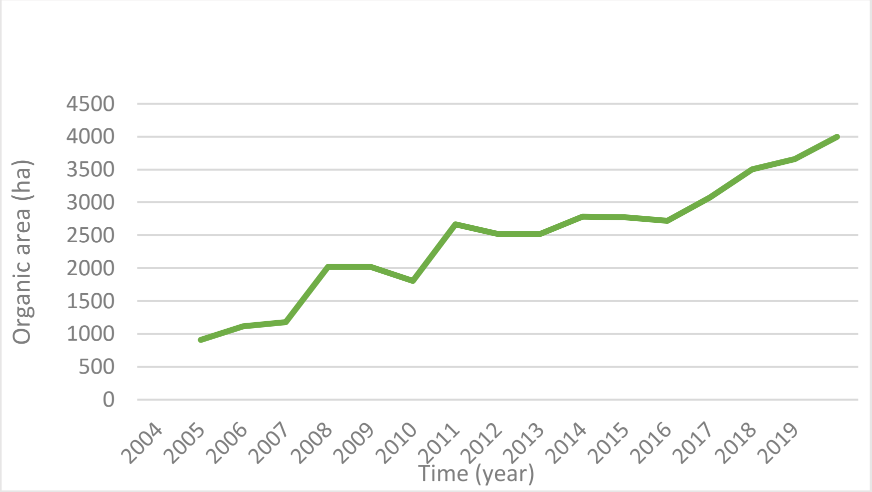

Figure 2- Evolution of the organic Vineyard area in Portugal since 2004 (FIBL, 2019)

In general, the principles of organic viticulture, according to the International Organisation of Vine and Wine (OIV-ECO 460-2012; OIV, 2012), are:

a) Maintain the ecosystems and fertility of soils on a long-term scale;
b) Increase biodiversity and the protection of natural resources;
c) Promote the use of ecological processes and cycles;

d) Minimize or eliminate external interventions and viticulture practices that require the use of chemical synthesis products;
e) Use organic products and processes in transformation and production processes, and trying to avoid all techniques having a considerable negative impact on the environment;
f) Excludes the use of genetically modified organisms and inputs issued from genetically modified organisms.

The organic viticulture system differs from the integrated one, has it moves away from synthetic fungicides towards Cu, S and plant resistance improvers. According to Neves (2012), organic viticulture refuses synthetic products for other products allowed by law. Moreover, organic fertilizers are promoted instead of mineral ones, and all chemical herbicides are not allowed. The use of species-rich cover crops adapted to the local soil can promote soil fertility by increasing organic matter, water use efficiency, and improving soil structure (Morrison-Whittle et al., 2017).

The change in the production systems is profound, establishing varieties adapted to better edaphoclimatic conditions, increasing soil fertility, and promoting biodiversity (Jones, 2013). Moreover, the use of natural products is intended to decrease the impact on the soil processes and biological activity. In the organic viticultural system, ecosystem self-sufficiency is sought in the organic matter cycle, using manure and/or composting as well as a herbaceous vegetation cover (Neves, 2012).

The combat of pests and diseases continues to be the most challenging problem to be dealt with when practicing organic viticulture. The strategies to control are based on regular analyses of the vine. The soil management techniques are equally essential, influencing plant nutrition and their capacity to resist pests and diseases (Neves, 2012).

2.3. Impact of organic management practices

Several soil characteristics and processes can be maintained and/or stimulated with organic management, thereby avoiding degradation of the vineyard system (Table 1). Nonetheless, for other parameters (e.g., Cu concentration in soil), the advantage of organic management is not clear (Table 1).

Table 1- Non-exhaustive review of the effects of organic viticulture on the soil parameters.

Soil parameters	Effect compared to integrated/ conventional management	References
Nutrients enrichment, nutrients cycling	Increase No difference	Hendgen et al. (2020) Döring et al. (2015)
Soil biological activity	Increase	Coll et al (2011); Freitas et al. (2011); Rienth et al. (2020)
Soil compaction	Increase	Coll et al. (2011)
Cu content in soil	Increase No difference	Hendgen et al. (2020) Coll et al. (2011); Karimi et al. (2021); Radić et al. (2014)
Metabolic quotient q CO_2	Decrease	Freitas et al. (2011); Rienth et al. (2020)
Organic C, total N, P, S, microbial biomass	Increase	Hendgen et al. (2020)
Microbial diversity and richness	Increase	Burns et al. (2016); Coll et al. (2011); Döring et al. (2019); Karimi et al. (2020)
Vegetation species density	Increase	Winter et al. (2018)

2.3.1. Fertilization and biological parameters

Nitrogen is essential to the soil. Fertilization is one of the key management practices in any cultivation. The most important source of N and other nutrients in organic farming is compost, although in some cases manures can be also used. The compost can enrich the soil with available N forms that plants will take up (Rienth et al., 2020). Moreover, available N in the superficial layer of the soil can be increased over some time under organic management due to the stimulation of soil nutrient cycling by compost application (Hendgen et al., 2020). As a result, the organic treatments showed a significantly higher available N content in the soil than the integrated management system (Döring et al., 2015). Nonetheless, for other macronutrients, there was no significant variation among management practices (Döring et al., 2015; Hendgen et al., 2020).

At biological level, organic vineyards showed higher cumulative soil respiration, microbial biomass C and biological activity, related to the C and P cycles (Coll et al., 2011; Freitas et al., 2011; Hendgen et al., 2020), a well as low qCO2 values (Rienth et al., 2020). Beyond the increase of microbial community, evaluated to microbial C, and their activity, several authors (Burns et al., 2016; Coll et al., 2011; Döring et al., 2019; Karimi et al., 2020) also reported higher microbial biodiversity. All these results can indicate a high microbial substrate-use efficiency and bioactive microbial community. Richness vegetation species density also has an increase (Winter et al., 2018).

2.3.2. Phytochemical treatments

Copper products are the oldest plant protection agents and represent an essential part of the plant protection strategy against *Plasmopara viticola*, (also known as grape downy mildew) in organic viticulture. However, this application can contribute to Cu accumulation in the soil, especially in the surface, and consequently negatively impact the total C cycle, enzyme activities, and biodiversity (Hendgen et al., 2020). Nonetheless, some studies (Coll et al., 2011; Radić et al., 2014) reported that organically managed vineyard soils from France, Croatia, and Germany did not have a higher Cu content than their conventional counterparts.

As a result of Cu enrichment in soil, the microbial community can vary over time, developing tolerant species in the community. For example, Karimi et al. (2021) found a microbial community with Cu tolerance up to doses of 400 kg Cu/ha. However, most microbial parameters were reduced by 30 to 60 % when doses ranging between 1600 and 8000 kg Cu/ha were applied, doses 400 to 2000 times higher than the doses applied by the winegrowers in one year (Karimi et al., 2021).

2.3.3. Vineyard management

Weed management is another crucial part of viticulture. Most winegrowers control ground vegetation using tillage, mulching, or herbicide application (Winter et al., 2018).

In experimental vineyards, organic viticulture promoted perennial species' growth compared to conventional farming, indicating a harmful impact of herbicide application on the establishment of perennial plant species. In addition, they found that organic plots host a more prosperous community of vascular plants, and vegetation species density was higher under organic farming (Winter et al., 2018).

Cover cropping is one of the most crucial viticulture practices associated with organic management. It is an essential ecological vineyard management tool. Cover cropping is widely used for organic vineyard management, bringing many improvements to soil properties. For instance, it prevents erosion, improves soil structure and porosity by limiting soil compaction, and manages soil moisture (Lopes et al., 2008). Also, plant species richness seemed to be higher under organic management, primarily due to cover crops and herbicide absence (Burns et al., 2016). The same author concluded that soil bacterial community structure, taxa abundances, and soil C and N pools differed by cover crop mix, suggesting an interaction among the cover crop type, soil resource pools, and microbial communities (Burns et al., 2016).

On the other hand, a decrease in pruning weight was due to cover cropping (Döring et al., 2015). The transpiration rates per unit leaf area of some cover crop species are about three times as high as those measured on the grapevine, leading to water stress. It can also affect soil moisture and, therefore, root development of the vines and physiological performance. One hypothesis is that the different types of cover crops used in this study influence water availability in the soil and thus physiological performance, growth, and vigour and cause interactions with the vines' root systems (Döring et al., 2015). Furthermore, mechanical weeding (typically used in organic viticulture) negatively impacts soil microbiome (Karimi et al., 2020).

Another alternative to the use of synthetic herbicide is mulching. Indeed, organic mulching is a sustainable agronomic practice, preventing soil erosion and improving general soil properties, including minimizing water loss through evaporation and run-off, improving water infiltration into the soil and water content, and increasing vineyard biodiversity (Buesa et al., 2021; Fraga et al., 2017). Additionally, organic mulching, such as pruning residues, could increase organic matter content and nutrients (in the long-run), water-holding capacity and inhibit the growth of weed.

The best possible agro-ecological pathway would be limiting tillage, significantly reducing synthetic pesticides, and promoting cover crops or mulching implementation (Karimi et al., 2021). Nevertheless, it is essential to know that stimulation of soil nutrient cycling by compost application, the implementation of cover crop mixtures with a wide range of species, the use of mulching and denial of mineral fertilizers and herbicides, as practiced in organic viticulture, take some years to make an impact on nutrient levels and microbial activity in the soil (Döring et al., 2019).

2.4. Relations between physico-chemical and biological characteristics of the soil

Physico-chemical indicators are still the most frequently used for soil quality assessment because they are paramount quantifiable indicators in an agronomical perspective, such as soil nutrient quantification (Heepngoen et al., 2021)

It is crucial to understand that the physico-chemical characteristics play a crucial role in soil biological communities and explain bacterial and fungal behaviour. Therefore, the evaluation of environmental parameters must always be coupled with the analysis of physico-chemical profiles when carrying out field studies to understand a particular soil better (Corneo et al., 2013). In general, the biological composition of a vineyard's soil can be affected by soil properties such as soil texture, pH, temperature, moisture, C, and N pools. For example, some dominant bacteria taxa, such as *Proteobacteria, Bacteroidetes, Gemmatimonadetes,* and *Firmicutes,* show a more dominant abundance in soils with lower C or N contents contrary to *Actinobacteria* (Burns et al., 2015).

Liang et al. (2019) identified pH, organic matter content, and total P content as the dominant factors for explaining a vineyard's soil bacterial community composition. Nonetheless, organic matter content explained the fungal community's most significant variation. Values of pH were also a crucial factor for the bacterial and fungal community variation on a vineyard’s soil (Bahram et al., 2018; Liang et al., 2019).

Biological properties also play crucial roles in maintaining and enhancing aboveground vine production, regulating soil C and various nutrient stocks. For instance, soil microbial diversity appears to play a pivotal role by controlling the cycling rate of soil C, N, K, and P (Li et al., 2020)

The biological component of the soil can be considered a vital part of the agricultural ecosystem, impacting soil productivity and product quality. In fact, the microbiome can influence the plant growth, health, and grape development (Di Giacinto et al., 2020). The rich diversity and variety of the microbiome can also ensure vital and productive soils and diminish negative management impacts (Hendgen et al., 2018). Moreover, soil microorganisms influence their local environment through pathogen suppression. They also impact the decomposition process that affects soil organic matter mineralization and contribute to the preservation and stability of soil organic matter (Hendgen et al., 2018).

Comprehending and analysing the functional biodiversity of the soil would allow better knowledge of their positive effect on the soil and vine and how they could be applied as bio-control agents, plant growth promoters, or bio-fertilizers (Pinto & Gomes, 2016).

The measured enzymatic activities shed light on the essential nutrients cycling (N, K, and P). This is in turn dependent on the efficiency of the soils microorganism's activity. Therefore, the functional soil biodiversity could be investigated by analysing the soil's enzymatic activities (Meena & Rao, 2021).

Enzyme activities can be valuable for evaluating soil quality since they not only participate in nutrient cycling but also play significant psychological functions in maintaining soil structure, degrading pollutants, and producing essential compounds for microorganisms and plants (Liang et al., 2019). It can be hypothesized that more metabolically active microorganisms will contribute to higher enzymatic activity in the vineyard system (Di Giacinto et al., 2020).

On the other hand, high enzyme activity indicates nutrient limitation, and a pattern of increasing enzyme activity with decreasing nutrient availability is sometimes found in soil (Di Giacinto et al., 2020). For example, Acid phosphatase activity commonly increases as P declines (Allison et al., 2007). However, negative relationships between nutrient availability and enzyme activity have not been consistently demonstrated (Song et al., 2021). Enzymes respond to soil management changes long before other soil quality indicator changes are detectable, allowing for more efficient management of the vineyard (Meena, 2021). It is crucial to understand what enzymes will be studied and their function as a biological parameter of the soil. Table 2 gives a concessive analysis of some enzymatic activities and their importance when evaluating the functional biodiversity of the soil.

Table 2- Enzymatic activities function on the soil.

Enzyme	Substances acted On	End product	Significance	Predictor of soil function
β –glucosidase	Carbon compounds	Glucose (Sugar)	Energy for microorganisms	Organic matter decomposition
Dehydrogenase	Carbon compounds	Hidrogen	Energy for microorganisms	Biological oxidation of organic matter
Urease	N under urea form	Ammonia and carbon dioxide	Plant available NH	Nutrient cycling
Cellulase	Cellulose	Glucose (Sugar)	Energy for microorganisms	Degradation of organic residues
Phosphatase	P	Phosphate	Plant available P	Nutrient cycling
Sulfatase	S	Sulfate	Plant available S	Nutrient cycling

Therefore, by understanding the physico-chemical properties of the soil and its correlation with soil biological components, it is possible to indicate more efficient and sustainable management practices for vineyard.

3. Material and Methods

3.1. Characterization of the studied areas

Two areas located in different demarcated wine regions were studied: Douro and Lisbon. The areas have different edaphoclimatic characteristics and different vineyard management practices, although in both cases the vineyards have an organic management. Therefore, data from different regions can allow a more complete understanding of the vineyards soil characteristics and its correlation and impact on the vine.

In Douro region, the study was developed in Quinta das Murças, in two plots with different planting system, while in Lisbon region it was in Monte D'Oiro.

3.1.1. Characterization of Quinta dos Murças and soil sampling

The Quinta dos Murças is located in the Douro region, namely in the Cima Corgo sub-region. The sub-region has vineyards in many different altitudes, exposures, slopes, and soils characteristics so, the terroirs of the vineyards are very distinctive between themselves (Prata-Sena et al., 2018). The total area is about 155 ha, of which ≈48 ha has the vine culture. Moreover, about 70 % of the vineyards are vertical vineyards and the rest are installed in narrow terraces, as it is the typical landscape from the Douro Region. Native varieties are the most planted in Quinta dos Murças (Table 3).

Table 3- Planted varieties in Quinta dos Murças.

Native Varieties	Other varieties
Touriga Nacional	Cabarnet Sauvignon
Touriga Franca	Sauvignon Blanc
Tinta Roriz	
Tinto Cão	
Tinta Amarela	
Tinta Barroca	
Sousão	

According to Kottek et al. (2006), the region has a temperate climate—Type C, sub-types Csa (temperate with hot and dry summer). The annual average precipitation is 700 mm with high amounts, mainly, from October to March (IPMA, 2021; Annex 1). The annual average temperature is 14.4º (IPMA, 2021; Annex 1). In the coldest months, January and February, the average temperature varies between 7.6°C and 12°C (IPMA, 2021; Annex 1) During the aestival period, the average values range between 21°C and 25°C, being the hottest months June, July, and August (IPMA, 2021; Annex 1).

Additionally, data between 2017 and 2020 from weather station from Quinta dos Murças was evaluated (Annex 1). These values of precipitation and temperature showed similar trends compared to the climatic normal values from 1931 to 1960 (Figure 3).

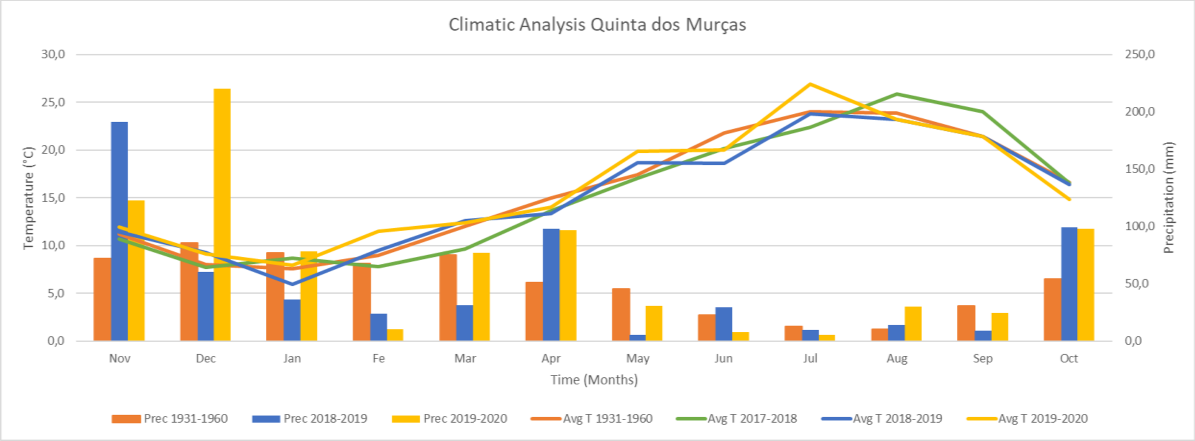

Figure 3- Quinta dos Murças climate normals from 1931-1960 and annual climatic analyses from recent years (2017-2020).

In the Douro Demarcated Region, most soils are classified as Anthrosols (WRB, 2015). These soils were developed on crushing schist and granitic rocks from the Douro Group formation (Pereira, 2020). In general, and according to the same author and WRB classification, these soils resulted from deep soil mobilizations with forced rock disaggregation or material mobilizations, resulting from cuts filled with consequent profile deepening, changes in the original soil horizons, and incorporation of fertilizers. The geological formation of the soils in the area is classified as metamorphic rocks (Sousa & Sequeira, 1989).

The topography in the Douro area is characterized by having high hillside slopes in almost all the sub-regions contributing to high risk of erosion (Figueiredo, 2015). Both the high stoniness and the slope create vineyard management limitations and low productivities (Figueiredo, 2015). In spite of the low productivity and this severe environment, the DDR is known for having a high-quality product, making the Douro region an outstanding example (Figueiredo, 2015; Jones, 2013).

Two different plots were evaluated in this studied area which is associated with the establishment system (Figure 4): P1, a vineyard established in a narrow terrace system while P2 was established in a vertical plating system. However, both plots have similar characteristics concerning the vineyard, namely the variety (Touriga Nacional), rootstock (1103P) and plant installation period (2010). The plot P1, with 0.54 ha, is between 120 m and 145 m and presents a slope of 40º while P2 has 0.60 ha, a slope of 30º and is located at a higher altitude (215-231 m).

Figure 4- Studied vineyards which were planted in different establishment systems. P1 on the left side and P2 on the right side of the figure.

The training system used in both plots is the Guyot system in a vertical shoot positioning system (VSP). The canes are tied to the trellis's lower wire in the Guyot system. In the terrace vineyard (P1), there is only one row of vines per terrace planted at 0.4 m of the edge of the embankment (Figure 4). The plant density in P1 is 2500 vines/ha with an average annual productivity of 3656 kg/ha. On the other hand, P2 was established as a vertical vineyard, which allows for easier mechanization and a higher plantation density (4165 vines/ha). Consequently, the productivity is being higher than in P1 (5497 kg/ha). Both systems presented a drip irrigation system. The deficit irrigation system is used which involves the supply of water below full crop evapotranspiration homogeneously along the growing season (Costa el al. 2012). Concerning the phytosanitary treatments, only Cu and S are applied (Annex 5).

In both vineyards, the grapevine growth stages can be identified using several descriptive, the Baggiolini scale (Baggiolini, 1952; Eichhorn & Lorenz, 1977) and BBCH scale (Lorenz et al. 1994). The grapevine growth stages in P1 were BBCH scale- **71** and Baggiolinu- **J.** In this growth stage, known as the fruit set, the young fruits begin to swell, and the remains of flowers are lost.

In the plot P1, eight composite samples were collected while in P2 were five composite samples. Each sample resulted from five sub-samples collected in the line until 20 cm depth and under the vine (Figure 5). This sampling methodology was designed in order to have a better representation of the heterogeneity of the plot, have the influence of the vine rhizosphere and obtain maximum potential of the biological parameters. All the sampling points were georeferenced (Figure 5).

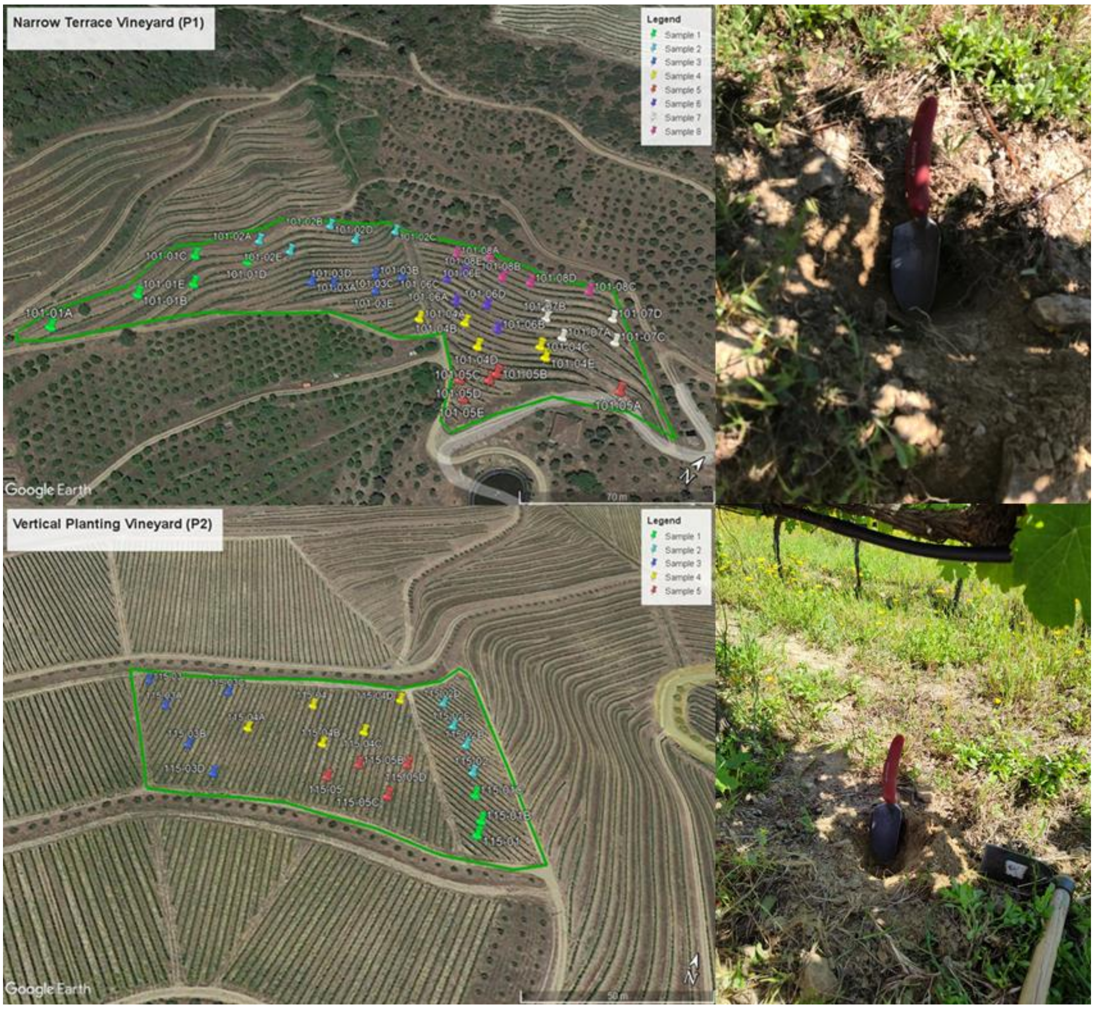

Figure 5- Localization of the subsamples collected in P1 and P2 and soil sampling in each plot.

3.1.2 Characterization of Monte D'Oiro and soil sampling

The Quinta do Monte D'Oiro is located in the Lisbon Wine Region, specifically in Alenquer. Alenquer is the most extensive wine producer in the region (IVV, 2017). The area of the "Quinta" reaches 42 ha, of which 30 ha planted with the vine culture. Many varieties are planted in Quinta do Monte D'Oiro (Table 4).

Table 4- Planted varieties in Quinta do Monte D'Oiro.

Native varieties	Other varieties
Touriga Nacional	Syrah
Tinta Roriz	Petit Verdot
Arinto	Viogner

According to Kottek et al. (2006), the region has a temperate climate—Type C, sub-types Csa (temperate with hot and dry summer). The annual average precipitation is 630 mm (IPMA, 2021; Annex 1). The annual average temperature is 15.1º reaching in the coldest months, January and February, values between 10°C and 12°C (IPMA, 2021; Annex 2). During the aestival period, the average values range between 18°C and 20°C, being the hottest months July, August, and September (IPMA, 2021; Annex 2).

Additionally, meteorological data between 2017 and 2020 and from weather station located in Quinta do Monte D’Oiro was evaluated (Annex 2). These values of precipitation and temperature showed similar trends compared to the climatic normal values from 1931 to 1960 (Figure 6).

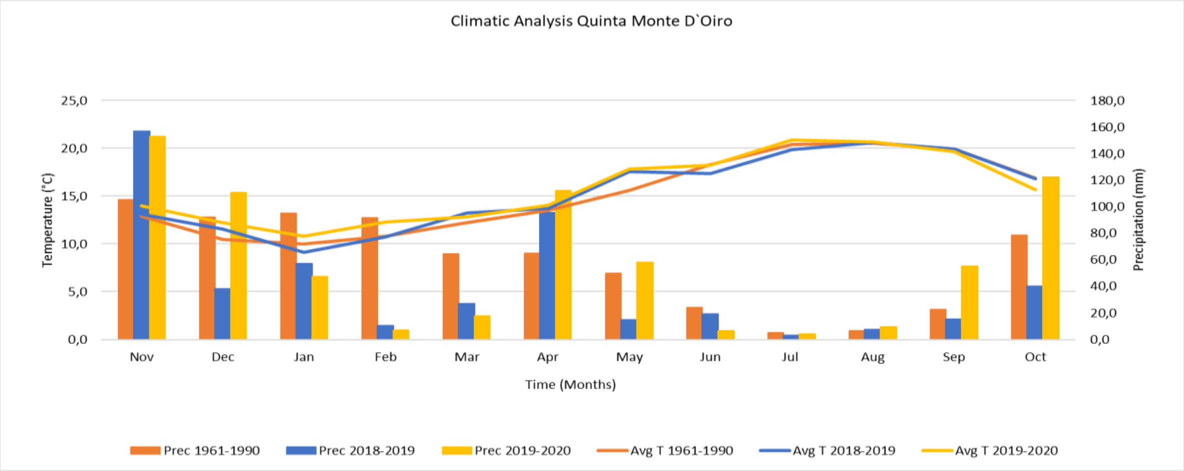

Figure 6- Quinta do Monte D'Oiro climate normals analysis from 1961-1990 and Annual climatic analyses from recent years (2018-2020).

According to Ferreira & Ferreira (2015), the soils from Quinta do Monte D'Oiro can be classified as Luvisols (WRB, 2015). Moreover, these soils were developed on the geological formation as sedimentary formation with limestone and marle nature in association with Jurassic sandstone (Zbyszewsk et al., 1966). The vineyard from the studied plot (P3) has a total area of 1.36 ha and was established in 2012 as a traditional planting system. The variety and rootstock in P3 are similar to Quinta dos Murças (Touriga Nacional, and rootstock 1103P). The training system used is the Guyot system in a vertical shoot positioning system.

The density of the plantation is 4166 plants/ha and the herbaceous vegetation in the inter-line is controlled, especially, by the tillage although pine oil is also used for weed management control (Figure 7, Annex 6). The average productivity is low (1900 kg/ha) despite of the high plant density. The grapevine growth stage was the same as in Quinta dos Murças (P1 and P2). No irrigation system exits in Quinta do Monte D’Oiro, contrarily to Quinta dos Murças. Concerning to phytosanitary treatments, Cu, S and some foliar fertilizers were applied in this vineyard.

Figure 7- Studied vineyard established in the traditional planting system (P3).

In this area, five composite soil samples were collected with the same methodology than in Quinta dos Murças plots: mixture of five soil sub-samples collected until 20 cm of depth and in the line. (Figure 8).

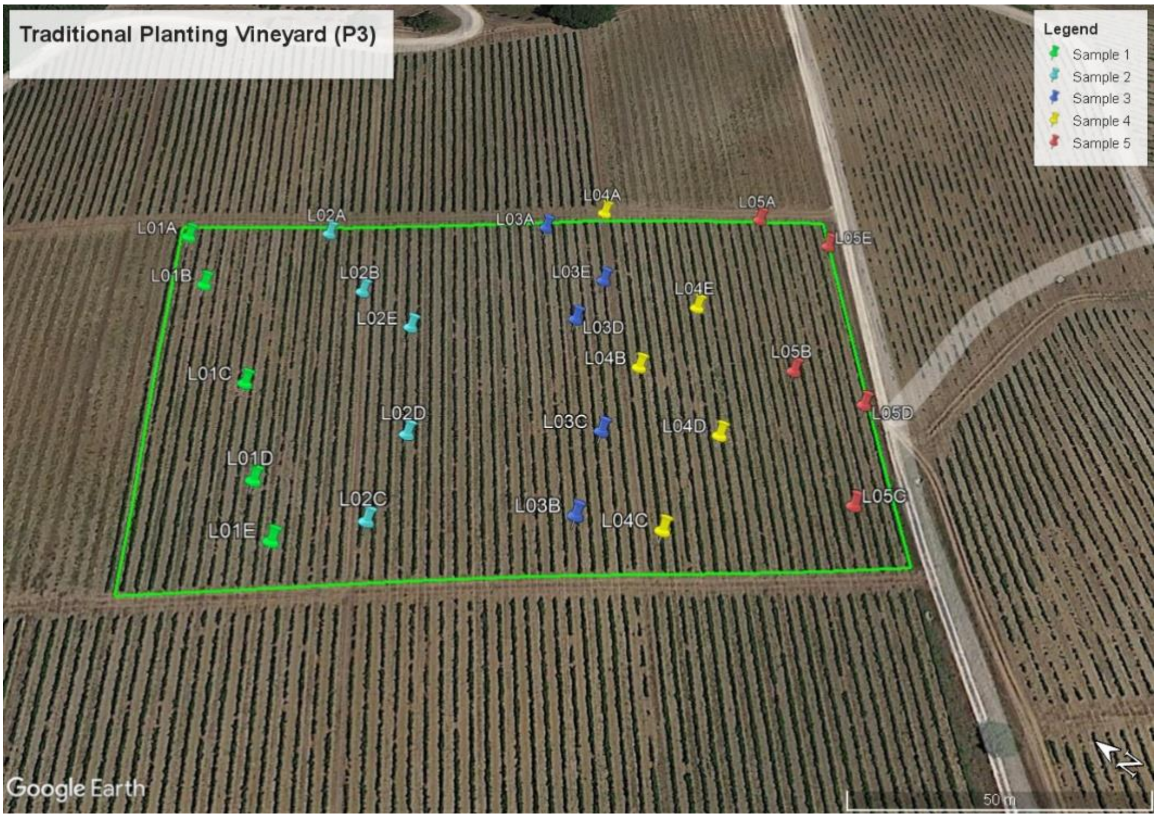

Figure 8- Localization of soil samples collected in P3.

3.2 Analytical methods

In the field, the composite soil samples were stored in plastic bags with their specific identification and transported to the Soil laboratory from Instituto Superior de Agronomia – Universidade de Lisboa. The soil samples were homogenized and divided in two fractions according to the type of analyses. Thus, a portion of each soil sample was kept fresh (4 °C) for the biological parameters, while the rest was air-dried for chemical properties. The fraction <2 mm was characterized for several physico-chemical and biological parameters following the standard soil methodologies (Table 5).

Table 5- Physico-chemical and biological analyses and respective methodologies used.

Laboratorial analyses		Analytical method	References
Physical	Coarse and fine fractions	Dry sieving	Botelho da Costa (1975)
	Soil moisture	Drying sample at 105°C for 24h	Botelho da Costa (1975)
Chemical	pH and electrical conductivity	H_2O (1:2.5 m:*V*) and determination by potentiometry	Smith et al. (2002)
	Organic C concentration	Wet dichromate oxidation and determination by titration	Springer & Klee (1954)
	Total N	Kjedahl method	McKenzie & Wallace (1954)
	Extractable P and K	Extraction by Egnér-Riehm method and elements determination by UV-VIS spectrophotometry and flame atomic absorption spectrophotometry	Egner et al (1960)
	Concentrations of Na, K, Ca, Mg, Fe, Cu, Mn and Zn in available fraction	Extraction by DTPA method and elements determination by flame atomic absorption spectrophotometry	Lindsay & Norvell (1978)
	Exchangeable non-acid cations	Extraction with ammonium acetate 1 M and elements determination by flame atomic absorption spectrophotometry	Madeira et al. (2003)
	Exchangeable acidity	Extraction with KCl 1 M and quantification by titration	Logan et al. (1985)
	Concentrations of Na, K, Ca, Fe, Cu, Mn and Zn in total fraction	Aqua regia digestion and elements determination by flame atomic absorption spectrophotometry	ISO 11466
Biological	β –glucosidase activity	The estimation of the released p-nitrofenol in a p-nitrophenyl-β-D-glucoside based on substrate, using UV-VIS spectroscopy	Eivazi & Tabatai (1988); Tabatabai (1982)
	Dehydrogenase activity	Triphenyltetrazolium chloride (TTC) Method, using UV-VIS spectroscopy	Thalmann (1968)
	Celulase activity	Determination of released reduction sugars in a Avicel based substract, using UV-VIS spectroscopy	Hope & Burns (1987)
	Acid phosphatase activity	Determination of released p-nitrofenol in a potassium p-nitrophenol based on substrate, using UV-VIS spectroscopy	Eivazi & Tabatai (1977); Tabatabai & Bremner (1970)
	Urease activity	Determination of released amoniacal N in a urea based on substrate, using UV-VIS spectroscopy	Kandeler & Gerber (1988)
	Sulfatase activity	Determination of of the release in a sulfate based on substrate, using UV-VIS spectroscopy	Tabatabai and Bremner (1970)

3.3 Statistical analysis

Data was analysed by one-way ANOVA and Tukey WSD test at $p \leq 0.05$, to compare the soil characteristics among studied plots. In the Douro region, the comparison was carried out between the two establishment systems: a vineyard in a narrow terrace system (P1) and vertical planting system (P2). This approach to the data allowed the evaluation of the effect of the establishment systems on the soil characteristics. Additionally, a comparison between regions, the vertical vineyard from the Douro (P2) and the traditional planting vineyard from the Lisbon region (P3) was carried out. Bivariate Pearson correlations were used to correlate soil parameters ($r < 0.85$; $r > -0.85$). All statistical analyses were performed using IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, NY, USA).

4. Results and discussion

In the Results and discussion section both the effect of establishment system on soil quality and the effect of vineyard location on soil quality were analysed.

4.1. Effect of establishment system on soil quality

In Quinta dos Murças, two different establishment systems were evaluated: one narrow terrace (P1) and a vertical plantation (P2). Both vineyards had the same variety, rootstock, management practices, and phytochemical treatments. However, the establishment system changes the density of the plantation, potential susceptibility to erosion and run-off.

The physical properties of the soil are crucial when assessing the quality of the soil since these affect other soil parameters, both biological and chemical level. The soil moisture and percentage of coarse fraction in both plots were similar (Table 6). The percentage of coarse elements in both plots can be associated to low pedogenesis process and weathering as well as the preparation of the soil for the vineyards by crushing the rocks (Pereira et al., 2020).

The pH values were neutral (Table 6), ideal for viticulture since it improves the availability of nutrients (Nicholas, 2004), although P1 presented a statistically higher pH. These values are higher than those usually found in soils from Douro Demarcated Region, which vary between 4.6 and 5.5 (Prata-Sena et al., 2018).

Table 6- Physico-chemical parameters of the soils collected in the studied vineyards with different establishment systems (average ± standard error; n= 8 and 5, respectively). Different letters indicate significant differences between establishment systems (p ≤ 0.05).

System of establishment of the vineyard		
Parameter	**Narrow terrace (P1)**	**Vertical planting (P2)**
Moisture (%)	9.00 ± 0.02 a	9.00 ± 0.01 a
Coarse fraction (%)	59.00 ± 0.08 a	51.00 ± 0.60 a
pH	7.48 ± 0.24 b	6.60 ± 0.26 a
Electrical conductivity (μS/cm)	64.77 ± 24.51 a	47.10 ± 6.56 a
SOC (g kg^{-1})	8.11 ± 1.73 a	9.43 ± 1.38 a
Total N (g kg^{-1})	0.44 ± 0.22 a	0.66 ± 0.04 a
C:N	21.10 ± 6.48 a	14.40 ± 7.44 a

Electrical conductivity (EC) can be considered an important indicator of soil health since indicates indirectly the elements in soil solution. In both plots, the EC was low and without significant differences between systems (Table 6), being non-saline soils according to Dahnke & Whitney (1988).

Independently of the studied system, low contents of organic C and N were observed (Table 6), a typical characteristic of soils from DDR (Jackson, 2008). Also, the C:N ratios were similar indicating in both cases a high mineralization of organic matter and release of N.

Soil cation exchange capacity (CEC) indicates the total amount of cations located in exchangeable complex. It is dependent on the content of organic matter and clay. Indirectly, this parameter can indicate the fertility of a soil since the elements located here correspond to available fractions. In general, the CEC values observed in both studied plots are considered low (Moyer et al., 2018), which can be associated to low organic matter amount (Table 6). Significant difference was obtained between plots, being the highest values in P1 (Table 7).

Table 7- Exchangeable cations concentrations (cmolc kg^{-1}), cation exchange capacity (CEC), percentage base saturation (BS%) and the ratio of various exchangeable cations of the soils in the studied vineyards with different establishment systems (average ± standard error; n= 8 and 5, respectively). Different letters indicate significant differences between establishment systems (p ≤ 0.05).

Parameter	Narrow terrace (P1)	Vertical planting (P2)
Ca (cmolc kg^{-1})	12.24 ± 1.46 b	1.29 ± 0.50 a
Mg (cmolc kg^{-1})	6.22 ± 1.13 b	1.07 ± 1.15 a
Na (cmolc kg^{-1})	0.09 ± 0.02 b	0.03 ± 0.03 a
K (cmolc kg^{-1})	0.03 ± 0.01 a	0.05 ± 0.01 a
Al (cmolc kg^{-1})	0.21 ± 0.02 a	0.22 ± 0.50 a

Parameter	Narrow terrace (P1)	Vertical planting (P2)
H (cmolc kg^{-1})	0.07 ± 0.03 a	0.07 ± 0.15 a
CEC (cmolc kg^{-1})	18.87 ± 1.69 b	2.73 ± 1.43 a
BS (%)	98.00 ± 0.01 b	86.00 ± 0.01 a
Ca:Mg	2.03 ± 0.45 a	2.60 ± 1.51 a
Ca:K	404.00 ± 120.02 b	28.17 ± 16.36 a
K:Mg	0.01 ± 0.00 a	0.14± 0.09 b

The percent of Base Saturation (BS%) represents the CEC sites occupied by non-acid exchangeable cations. In this scenario, the contribution of acid cations is very low, what agrees with the pH values. The BS% in P1 was significantly higher than in P2 (Table 7), although Ca was the dominant cation in exchangeable complex in both cases. These lower values of non-acid cations in P2 can be explained by the higher runoff that can occur on a vertical vineyard, which will cause the leaching of elements (Queiroz, 2008). On the other hand, some terraces can act as drainage for others, which accumulate particulate on recipient terraces being lower the intensity of erosion and runoff (Queiroz, 2008). The higher BS% as well as the higher content of non-acid cations, mainly Ca and Mg, could be due to a higher mineralogical weathering in these terraces, because of the fragmentation of the rocks.

The ratio between different exchange cations allows evaluating imbalances of cations that could affect plant growth. The Ca:Mg ratio is a determinant factor for the absorption of calcium and magnesium by plants, whose optimal values range between 2 and 5 (Van Schoor et al., 2000). A ratio of 2 to 5 is the benchmark to ensure healthy soil and optimum agriculture production. Thus, both plots are within the benchmark ratio (Table 7). The Ca:K and K:Mg ratios showed significant differences between establishment systems. The suitable Ca:K range is less than 30, while the K:Mg ratio is between 0.2 - 0.35 for most crops (Espinoza et al., 2019). However, both ratios indicate that K is a deficient cation in the CEC (Table 7), making it harder for the vine roots root system to uptake potassium (Espinoza et al., 2019).

The total concentration of elements (except Fe and Ca) reached the highest values in P1 (Table 8). Nonetheless, the elements in available fraction corresponded, in both the cases, to a small percentage of total amounts.

Independently of nutrients type and need to the plant, the available concentrations were very small, and no differences were obtained between the establishment systems (except Ca and Mg) in available fraction were similar (Table 8). This fact, together with concentrations of organic C and N, indicate a low fertility of the soils from vineyards from Douro and, consequently, a possible limitation of plant development and productivity. For Ca and Mg, P1 soils showed the highest concentrations (Table 8).

Table 8- Nutrient concentrations (g kg^{-1}) in available and total fraction of the soils collected in the studied vineyards with different establishment systems (average ± standard error; n= 8 and 5, respectively). Different letters in same parameter (element and soil fraction) indicate significant differences between establishment systems (p ≤ 0.05).

	Narrow terrace (P1)		Vertical planting (P2)	
Nutrient	**Available**	**Total**	**Available**	**Total**
Fe	0.08 ± 0.02 a	46.79 ± 2.71 a	0.11 ± 0.01 a	44.35 ± 1.50 a
Mn	0.03 ± 0.01 a	0.87 ± 0.19 b	< DL a	0.21 ± 0.28 a
Zn	0.03 ± 0.01 a	0.08 ± 0.01 b	< DL a	0.06 ± 0.01 a
Cu	0.01 ± 0.01 a	0.25 ± 0.26 a	< DL a	0.52 ± 0.29 b
Ca*	2.45 ± 0.29 b	2.22 ± 0.43 a	0.26 ± 0.10 a	0.99 ± 0.51 a
Mg	0.93 ± 0.20 b	2.22 ± 0.48 b	0.18 ± 0.73 a	0.99 ± 0.31 a
K	0.05 ± 0.02 a	0.37 ± 0.10 b	0.07 ± 0.13 a	0.15 ± 0.13 a
P	0.08 ± 0.03 a	559.46 ± 63.51 b	0.13 ± 0.12 a	0.60 ± 0.37 a

*The available Ca presented in the table corresponds to the Ca determined to CEC

Soil enzymatic activities are used as indicators of soil microbial community functioning associated to nutrient cycling and general state of community (Di Giacinto et al., 2020). In general, the several studied enzymatic activities were small, and no significant differences were obtained according to establishment system (Table 9). This fact agrees with the similarity that also exists for physico-chemical characteristics (Tables 6, 7 and 8). Moreover, in general, no direct relations were obtained among physico-chemical characteristics and enzymatic activities.

Acid phosphatase (PHO) is a broad group of enzymes involved in the phosphorus cycle, specifically in the conversion of organic P into inorganic P which is taken up by roots, and is strongly connected to soil fertility (Di Giacinto et al., 2020). The Urease (URE) activity is involved in urea hydrolysis to carbon dioxide and ammonia, which is a form of N available for plants (Di Giacinto et al., 2020). Both enzymes contribute to the availability of these nutrients in the soils. Nonetheless, although P and N concentrations are small (Table 6, 8) in the soils from both studied plots, these enzymes are not being stimulated. This fact can be associated to previous additions of P and N fertilizers, which can lead to a decrease of these activities. For PHO activity, neutral values of pH in the soils (Table 6) can justified the small values (r = -0.87; Annex 3). However, for urease, no significant relations were obtained with studied physico-chemical characteristics.

Table 9- Enzymatic activities of the soils collected in studied vineyards with different establishment systems (average ± standard error; n= 8 and 5, respectively). Different letters indicate significant differences between establishment systems (p ≤ 0.05).

Establishment of the vineyard		
Enzymatic activities	**Narrow terrace (P1)**	**Vertical planting (P2)**
Acid phosphatase (μmol NP g^{-1} h^{-1})	0.62 ± 0.14 a	1.05 ± 0.13 a
β-glucosidase (μmol NP g^{-1} h^{-1})	0.26 ± 0.05 a	0.51 ± 0.09 a
Cellulase (μmol G LU g^{-1} $16h^{-1}$)	0.15 ± 0.11 a	0.41 ± 0.33 a
Urease (μmol NH4-N g^{-1} $2h^{-1}$)	0.15 ± 0.30 a	0.34 ± 0.24 a
Sulfatase (μmol TPF g^{-1} h^{-1})	0.45 ± 0.30 a	0.65 ± 0.24 a
Dehydrogenase (μg TPF g^{-1} h^{-1})	3.51 ± 2.32 a	5.79 ± 3.55 a

The activities of β-glucosidase (GLU) and cellulase provide information on the carbon cycle. Cellulase is an enzyme that catalyses the degradation of cellulose (Deng & Tabatabai, 1994) while β-glucosidase catalyses the hydrolysis of sugars, resulting in β-linked monosaccharides. The final product of GLU reaction is the glucose, a carbon source for soil microorganisms (Baddam et al., 2016). Both enzymes presented small values indicating a low functioning of the C cycle. Low contents of organic matter or absence of lignified materials can justify these results (Di Giacinto et al., 2020; Karimi et al., 2021).

4.2. Effect of vineyard location on soil quality

A comparison between vineyards in different regions can also give important information on regional variability of soil characteristics, potentially associated with differences in the vineyard management practices. However, each region englobes other factors that could influence the vineyard soils, such as edaphoclimatic characteristics, vineyard establishment system, topography, and management practices, that were not studied. Therefore, this statistical analysis is not as robust as the previous one. The plots analysed and compared were P2, located in Quinta dos Murças (Douro Wine Region), and P3, a traditional planting vineyard located in Quinta do Monte D'Oiro (Lisbon Wine Region).

Physical characteristics of the soils (moisture and % of coarse elements) were similar between the plots (Table 10). Despite of the plot P2 has an irrigation system, this fact is not reflected in the data.

Table 10- Physico-chemical parameters in the soils collected in the studied vineyards located in different regions (average ± standard error; n= 5). Different letters indicate significant differences between regions according to Tukey b post-hoc tests at $p \leq 0.05$.

Parameter	Vertical planting from Douro (P2)	Traditional planting from Lisbon (P3)
Moisture (%)	9.00 ± 0.01 a	10.00 ± 0.00 a
Coarse fraction (%)	51.00 ± 0.60 a	51.00 ± 0.12 a
pH	6.60 ± 0.26 a	6.26 ± 0.72 a
CE (μS/cm)	47.10 ± 6.56 a	153.93 ± 94.98 b
SOC (g kg^{-1})	9.43 ± 1.38 a	14.58 ± 1.82 b
N (g kg^{-1})	0.66 ± 0.04 a	0.99 ± 0.15 b
C:N	14.40 ± 7.44 a	14.86 ± 7.28 a

pH values in both studied plots are considered neutral (and similar) and ideal for the vine culture (Prata-Sena et al., 2018). For other chemical characteristics, soils from Lisbon presented the highest values (Tables 10 and 11). The SOC and N value in P3 can be considered high values for agriculture soils. The difference in SOC and N in P3 can be related to the more frequent compost applications (Annex 5). Source of N seems to be correlated to SOC (r= 0.90).

The CEC in P3 is considered a high value (Moyer et al., 2018). The higher CEC value can be associated to higher SOC content in this plot compared to P2. Percentage of base saturation (BS%) is high in both areas, consistent with the pH values (Moyer et al., 2018). Calcium is allways the dominant non acid cation in the exchangeable complex. The Douro Region (P2) has a lower BS% that can be related to a higher lixiviation (Table 11). The higher lixiviation can be caused by the run-off associated with the vertical establishment system used in P2 (Queiroz, 2008).

Table 11- Exchangeable cations concentrations (cmolc kg^{-1}), cation exchange capacity (CEC), percentage base saturation (BS%) and the ratio of various exchangeable cations of the soil collected in studied vineyards located in different regions (average ± standard error; n= 5). Different letters indicate significant differences between regions ($p \leq 0.05$).

Parameter	Vertical planting from Douro (P2)	Traditional planting from Lisbon (P3)
Ca (cmolc kg^{-1})	1.29 ± 0.50 a	10.25 ± 7.15 b
Mg (cmolc kg^{-1})	1.07 ± 1.15 a	3.82 ± 2.49 b
Na (cmolc kg^{-1})	0.03 ± 0.03 a	0.06 ± 0.03 a
K (cmolc kg^{-1})	0.05 ± 0.01 a	0.07 ± 0.05 b
Al (cmolc kg^{-1})	0.22 ± 0.50 a	0.26 ± 0.04 a

Parameter	Vertical planting from Douro (P2)	Traditional planting from Lisbon (P3)
H (cmolc kg^{-1})	0.07 ± 0.15 a	0.07 ± 0.08 a
CEC (cmolc kg^{-1})	2.73 ± 1.43 a	19.27 ± 7.01 b
BS (%)	86.00 ± 0.01 a	98.00 ± 0.01 b
Ca:Mg	2.60 ± 1.51 a	6.08 ± 3.08 b
Ca:K	28.17 ± 16.36 a	107.40 ± 42.72 a
K:Mg	0.14± 0.09 b	0.06 ± 0.01 a

Contrarily to P2, the Ca:Mg ratio in P3 is over the benchmark ratio to ensure healthy soil and optimum agriculture production, showing a magnesium deficiency in the soil (Table 11) (Van Schoor et al., 2000). In the two regions, both K:Mg and Ca:K ratios indicated that K is a deficient cation in the CEC (Table 11), making it harder for the vine roots root system to uptake potassium (Espinoza et al., 2019). Moreover, the deficiency in K can lead to defoliation and a reduction in the vine vigour and crop yield (Moyer et al., 2018).

In total fraction, P2 presented higher concentrations of Fe and Cu, while in P3, Mg and K reach the highest values (Table 12). This tendency was not seen in the available fraction. In fact, Quinta do Monte D'Oiro (P3) has a high availability of many nutrients (Fe, Mn, and Cu) what explains the higher EC (Table 10). Moreover, available P and Mn were correlated with their total nutrient concentration (Annex 4), while the source of available K can be SOC (r= 0,92; Annex 4).

Table 12- Available and total Nutrient concentrations (g kg^{-1}) of the soils collected in the studied vineyards located in different regions (average ± standard error; n= 5). Different letters indicate significant differences between regions, according to Tukey b post-hoc tests at p ≤ 0.05.

	Vertical Planting from Douro (P2)		Traditional Planting from Lisbon (P3)	
Nutrient	**Available**	**Total**	**Available**	**Total**
Fe	0.11 ± 0.01 a	44.35 ± 1.50 b	0.17 ± 0.05 b	34.06 ± 5.98 a
Mn	0.01 ± 0.01 a	0.21 ± 0.28 a	0.06 ± 0.15 b	0.35 ± 0.08 a
Zn	< DL a	0.06 ± 0.01 a	< DL a	0.05 ± 0.01 a
Cu	0.01 ± 0.01 a	0.52 ± 0.29 b	0.03 ± 0.01 b	0.09 ± 0.01 a
Ca*	0.26 ± 0.10 a	0.99 ± 0.51 a	2.05 ± 1.43 a	4.06 ± 4.81 a
Mg	0.18 ± 0.73 a	0.99 ± 0.31 a	0.26 ± 0.49 a	4.06 ± 4.81 b
K	0.07 ± 0.13 a	0.15 ± 0.13 a	0.11 ± 0.31 a	0.21 ± 0.03 b
P	0.13 ± 0.12 a	0.60 ± 0.37 a	0.04 ± 0.70 a	0.24 ± 0.03 a

In general, soils from P3 presented a more active microbial community with higher values of PHO, GLU and SUL compared to P2. Although without a significant correlation, the low concentrations of available phosphorus in P3 can justify the stimulation of PHO activity. Some studies support the results, as the acid phosphatase enzyme activity was negatively correlated with the concentration of P in the soil (Allison et al., 2007; Baddam et al., 2016).

Table 13- Enzymatic activities of the soils collected in the studied vineyards located in different regions (average ± standard error; n= 5). Different letters indicate significant differences between regions according to Tukey b post-hoc tests at p ≤ 0.05.

Enzymatic activities	Vertical planting from Douro (P2)	Traditional planting from Lisbon (P3)
Acid phosphatase (µmol NP g^{-1} h^{-1})	1.05 ± 0.13 a	1.51 ± 0.56 b
β-glucosidase (µmol NP g^{-1} h^{-1})	0.51 ± 0.09 a	1.03 ± 0.19 b
Cellulase (µmol G LU g^{-1} $16h^{-1}$)	0.41 ± 0.33 a	0.33 ± 0.06 a
Urease (µmol NH4-N g^{-1} $2h^{-1}$)	0.34 ± 0.24 a	0.60 ± 0.42 a
Sulfatase (µmol TPF g^{-1} h^{-1})	0.65 ± 0.24 a	1.11 ± 0.42 b
Dehydrogenase (µg TPF g^{-1} h^{-1})	5.79 ± 3.55 a	9.46 ± 4.13 a

Higher SOC contents in P3 soil can justified by the β-glucosidase activity (r=0.86) (r= 0.92; Annex 4). The relation between SOC and GLU is backed by various studies (Baddam et al., 2016; Bandick & Dick, 1999). Differences between N concentrations were not reflected in urease activity.

Differences in SUL activity can be due to the different dosages of S applied in each region. Sulfatase enzymes are “sulfate starvation-induced” proteins produced by microorganisms, during S starvation (Gardner & Senwo, 2019) (Annex 5, 6).

5. Conclusions

This work's main goal was to characterize the soil of various organically managed plots and assess the soil quality concerning biological and physico-chemical properties. The data showed that the soil from Quinta do Monte D'Oiro is well suited for the vineyard culture. Quinta do Monte D'Oiro had more available nutrients and had high levels of organic carbon and total N. Moreover, all enzymatic activities had higher values, reflecting a more active microbial community. Quinta dos Murças has a good pH but with low availability for most of the nutrients, and a poor SOC and total N levels. These results in the Douro plots seem to contribute to a poorer functional biodiversity, when compared with the soils of Quinta do Monte D’Oiro.

The second goal of this study was to analyse the differences in soil characteristics between different vineyards in the same region, a vineyard established on a terrace system, and a vertical planting vineyard. In general, the established system did not affect the chemical and biological soil quality, except for pH, some cation exchangeable complex characteristics (exchangeable Ca, Mg, Na and CEC). Between regions, there were significant differences in some soil enzymatic (except in Dehydrogenase and Urease) and almost all physico-chemical parameters, such as soil organic carbon, total N, cation exchange capacity characteristics and nutrients in the available fraction (Cu, Fe, Mn, Zn and P).

This study reveals explanatory data for complete soil characterization and analysis of organically managed vineyards. The lack of individual correlations between biological and chemical parameters can be associated with a combined effect of chemical characteristics on enzymatic activities. More studies are needed.

The differences in the quality of the soils seem to be uncorrelated with the vineyard's performance, as measured by productivity. For example, Quinta do Monte D'Oiro had a much lower productivity than the plot in Quinta dos Murças. Therefore, future studies explaining the direct impact of the soil characteristics on the vine itself should also be conducted. It would be interesting to understand how different soil properties impact the vine's sanity, productivity, and grapes' quality.

Moreover, it would be interesting to understand the impact of irrigation on soil quality and the vineyard’s response to water availability, analysing parameters such as root development, vegetative growth, productivity and grape quality.

6. References

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7. Annexes

Annex 1- Climatic data from the Weather station located in Quinta dos Murças.

Period	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Prec 1931-1960	71.9	85.9	77.2	67.7	75.1	51.3	45.5	22.8	12.5	10.4	30.4	53.9
Prec 2017-2018	24.4	96.8	65.4	73.8	260.4	88.0	72.8	113.6	6.2	0.0	5.6	36.2
Prec 2018-2019	191.4	60.2	36.2	24.0	31.6	97.8	5.6	29.6	9.6	14.2	8.8	99.0
Prec 2019-2020	123.0	220.6	78.2	10.6	77.2	97.0	30.6	7.6	5.4	30.2	24.6	98.2
Days P>0 2017-2018	6.0	12.0	9.0	9.0	25.0	14.0	9.0	10.0	2.0	0.0	1.0	8.0
Days P>0 2018-2019	19.0	8.0	8.0	3.0	5.0	18.0	4.0	8.0	2.0	4.0	5.0	11.0
Days P>0 2019-2020	27.0	14.0	13.0	4.0	11.0	18.0	10.0	2.0	2.0	3.0	5.0	12.0
Max T 2017-2018	17.6	12.9	13.5	13.7	14.6	19.9	24.1	27.1	29.5	34.8	33.2	23.8
Max T 2018-2019	15.6	13.0	11.0	16.6	19.8	19.5	26.1	25.3	31.9	31.2	29.2	22.9
Max T 2019-2020	15.6	12.7	11.5	17.2	18.3	19.7	27.9	26.9	35.7	31.1	29.5	21.1
Avg T 1931-1960	11.2	8.0	7.6	9.0	12.0	15.0	17.4	21.8	24.0	23.9	21.4	16.6
Avg T 2017-2018	10.7	7.7	8.7	7.8	9.7	13.6	17.1	20.2	22.4	25.9	24.0	16.5
Avg T 2018-2019	11.4	9.2	6.0	9.5	12.7	13.3	18.7	18.6	23.8	23.2	21.4	16.4
Avg T 2019-2020	11.9	9.1	7.9	11.5	12.4	14.0	19.9	20.0	26.9	23.2	21.5	14.8
Min T 2017-2018	5.3	3.8	4.8	2.9	6.0	8.5	11.2	14.7	16.2	17.4	16.1	10.5
Min T 2018-2019	8.5	6.5	2.5	3.9	6.1	8.4	11.3	12.4	16.7	16.2	14.8	11.5
Min T 2019-2020	9.1	6.5	5.2	7.1	7.4	9.8	13.4	13.5	18.6	16.2	14.5	10.0

Annex 2- Climatic data from the Weather station located in Quinta do Monte D'Oiro.

Period	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Prec 1961-1990	105.5	92.3	94.9	91.7	64.3	64.7	49.8	24.2	5.2	6.6	22.4	78.3
Prec 2018-2019	156.8	38.3	57.3	10.3	26.8	95.5	15.0	19.0	3.3	7.8	15.5	40.0
Prec 2019-2020	152.9	110.6	47.5	7.3	17.8	112.1	58.3	6.5	4.0	9.3	55.0	122.5
Days P>0 1961-1990	13.7	12.9	14.9	14.7	11.6	12.4	8.7					
Days P>0 2018-2019	21.0	8.0	12.0	4.0	4.0	19.0	5.0					
Days P>0 2019-2020	29.0	13.0	12.0	6.0	12.0	18.0	8.0					
Max T 1961-1990	17.6	14.7	14.2	14.9	16.8	18.1	20.3					
Max T 2018-2019	17.3	17.1	15.3	17.6	20.0	18.1	23.3					
Max T 2019-2020	17.1	17.0	15.6	18.5	17.8	17.9	22.8					
Max T 2020-2021	18.9	15.1	13.8	16.8	18.8	19.7	19.6					
Avg T 1961-1990	12.9	10.5	10.0	10.8	12.2	13.5	15.6	18.3	20.4	20.5	19.9	16.9
Avg T 2018-2019	13.1	11.5	9.1	10.7	13.2	13.7	17.6	17.4	19.9	20.6	19.9	16.8
Avg T 2019-2020	14.0	12.2	10.8	12.3	12.9	14.1	17.8	18.2	20.9	20.6	19.7	15.7
Min T 1961-1990	t	6.5	5.8	6.7	7.6	8.8	10.9					
Min T 2018-2019	9.0	6.6	4.1	4.9	6.6	8.9	11.9					
Min T 2019-2020	10.8	7.8	6.4	6.7	7.8	9.8	13.0					
Acum ColdHours 2018-2019	67.0	197.0	416.0	604.0	713.0	743.0	746.0					
Acum ColdHours 2019-2020	28.0	148.0	320.0	437.0	522.0	557.0	557.0					
Acum T>7.3 2018-2019	174.4	305.7	375.3	473.4	657.5	849.3	1167.7					
Acum T>7.3 2019-2020	199.9	352.8	465.5	609.4	781.5	984.2	1311.0					
Acum T>7.3 2020-2021	202.4	325.3	415.6	576.7	735.2	966.7	1173.2					
Acum T>10 2018-2019	94.9	145.7	173.8	210.0	310.4	421.2	655.9					
Acum T>10 2019-2020	120.8	196.0	252.9	319.6	410.3	533.9	777.0					
Acum T>10 2020-2021	121.4	177.0	228.9	314.4	389.9	540.4	676.7					

Annex 3- Bivariate Pearson correlations table for all factors analysed, between plots with different establishments

P1 VS P2		Physicochemical				Available Nutrients									Total Nutrients									Exchange Cations						Enzymatic Activities						Physical Prop.	
		pH	CE	SOC	N	Fe	Mn	Zn	Cu	Mg	Na	K	P	K	Fe	Mn	Zn	Cu	Ca	Mg	Na	K	P	Ca	Mg	Na	K	Al	CTC	Phosphatase	β-glucosidase	Cellulase	Urease	Sulfatase	Dehydrogenase	Moisture	Coarse elements
Physicochemic	pH	**1.00**																																			
	CE	0.60	**1.00**																																		
	SOC	-0.16	0.53	**1.00**																																	
	N	-0.28	0.49	0.75	**1.00**																																
Available Nutrients	Fe	-0.67	-0.17	0.17	0.56	**1.00**																															
	Mn	0.71	0.26	-0.41	-0.42	-0.39	**1.00**																														
	Zn	0.59	0.73	0.17	0.36	-0.05	0.53	**1.00**																													
	Cu	0.42	0.69	0.19	0.26	-0.04	0.09	0.62	**1.00**																												
	Mg	0.80	0.24	-0.50	-0.58	-0.56	**0.89**	0.34	0.11	**1.00**																											
	Na	-0.02	-0.23	-0.42	-0.27	-0.10	-0.09	-0.39	-0.09	0.23	**1.00**																										
	K	-0.24	0.33	0.64	0.69	0.42	-0.37	0.31	0.29	-0.63	-0.55	**1.00**																									
	P	-0.07	0.03	0.03	0.38	0.17	-0.32	-0.01	-0.05	-0.33	0.19	0.18	**1.00**																								
	K	-0.47	0.03	0.48	0.58	0.35	-0.58	-0.18	-0.10	-0.76	-0.34	0.70	0.42	**1.00**																							
Total Nutrients	Fe	0.63	0.62	0.15	0.21	-0.21	0.61	0.80	0.30	0.53	-0.14	0.10	-0.15	-0.41	**1.00**																						
	Mn	0.84	0.42	-0.34	-0.38	-0.56	**0.90**	0.50	0.16	**0.95**	0.05	-0.50	-0.31	-0.61	0.64	**1.00**																					
	Zn	0.82	0.79	0.12	0.04	-0.50	0.70	0.67	0.47	0.71	-0.01	-0.15	-0.17	-0.36	0.74	0.84	**1.00**																				
	Cu	-0.50	-0.62	0.00	-0.05	0.01	-0.54	-0.42	-0.30	-0.50	0.11	0.00	0.04	0.11	-0.34	-0.56	-0.60	**1.00**																			
	Ca	**0.90**	0.55	-0.18	-0.20	-0.55	0.62	0.50	0.39	0.76	0.16	-0.40	0.13	-0.58	0.59	0.76	0.77	-0.47	**1.00**																		
	Mg	0.66	0.09	-0.47	-0.67	-0.63	0.74	0.06	-0.02	**0.92**	0.34	-0.82	-0.38	-0.77	0.28	0.84	0.60	-0.33	0.68	**1.00**																	
	Na	0.69	0.07	-0.48	-0.76	-0.74	0.65	0.01	0.04	0.84	0.26	-0.67	-0.29	-0.73	0.26	0.72	0.51	-0.31	0.71	**0.88**	**1.00**																
	K	0.61	0.13	-0.36	-0.60	-0.61	0.51	-0.05	0.05	0.80	0.45	-0.81	-0.35	-0.70	0.14	0.71	0.53	-0.27	0.62	**0.94**	0.77	**1.00**															
	P	0.17	0.05	-0.16	0.25	0.09	-0.02	0.21	0.02	-0.04	0.23	-0.02	**0.91**	0.11	0.06	-0.03	0.00	0.02	0.36	-0.12	-0.13	-0.12	**1.00**														
Exchange Cations	Ca	**0.87**	0.48	-0.30	-0.44	-0.62	0.66	0.37	0.46	**0.86**	0.23	-0.51	-0.32	-0.63	0.41	0.84	0.77	-0.49	0.80	0.82	0.74	**0.85**	-0.06	**1.00**													
	Mg	0.77	0.21	-0.53	-0.60	-0.50	0.66	0.32	0.32	0.84	0.14	-0.59	-0.34	-0.73	0.29	0.77	0.51	-0.39	0.70	0.79	0.71	0.79	-0.03	**0.91**	**1.00**												
	Na	0.69	0.26	-0.39	-0.59	-0.55	0.53	0.06	0.28	0.80	0.35	-0.70	-0.30	-0.68	0.12	0.70	0.55	-0.47	0.71	**0.87**	0.81	**0.93**	-0.10	**0.91**	**0.88**	**1.00**											
	K	-0.41	0.27	0.68	0.72	0.43	-0.52	0.13	0.14	-0.74	-0.50	**0.93**	0.18	0.83	-0.09	-0.57	-0.24	0.00	-0.56	**-0.86**	-0.77	-0.80	-0.09	-0.59	-0.68	-0.72	**1.00**										

	Al	-0.15	0.03	0.09	0.08	-0.08	-0.03	-0.15	-0.08	-0.15	-0.28	0.16	-0.20	0.50	-0.13	0.02	0.09	-0.05	-0.28	-0.11	-0.09	-0.19	-0.40	-0.14	-0.30	-0.21	0.29	**1.00**									
	CTC	0.57	0.05	-0.58	-0.56	-0.45	0.59	0.03	0.03	0.85	0.72	-0.75	-0.13	-0.73	0.30	0.71	0.51	-0.30	0.64	0.85	0.75	0.82	0.10	0.75	0.68	0.77	-0.80	-0.26	**1.00**								
Enzymatic Activities	**Fosfatases Ácidas**	**-0.87**	-0.27	0.50	0.57	0.64	-0.74	-0.41	-0.32	-0.79	0.02	0.27	0.16	0.43	-0.39	-0.76	-0.58	0.41	-0.69	-0.62	-0.72	-0.52	-0.06	-0.79	-0.80	-0.63	0.44	0.02	-0.56	**1.00**							
	Glucosidases	-0.76	-0.22	0.40	0.59	0.61	-0.64	-0.07	-0.12	-0.84	-0.25	0.51	0.36	0.48	-0.28	-0.79	-0.59	0.40	-0.62	-0.81	-0.76	-0.80	0.18	**-0.86**	-0.78	-0.80	0.58	-0.09	-0.74	0.80	**1.00**						
	Celulases	-0.40	-0.17	0.20	0.28	0.68	-0.38	-0.13	-0.07	-0.42	-0.15	0.48	0.00	0.14	-0.06	-0.52	-0.53	0.08	-0.37	-0.54	-0.42	-0.49	-0.09	-0.46	-0.30	-0.40	0.36	-0.32	-0.39	0.35	0.33	**1.00**					
	Ureases	-0.48	-0.03	0.28	0.49	0.27	-0.41	-0.10	0.05	-0.59	-0.09	0.38	0.40	0.67	-0.27	-0.47	-0.17	0.28	-0.39	-0.53	-0.49	-0.56	0.17	-0.55	-0.73	-0.59	0.45	0.59	-0.47	0.46	0.53	-0.22	**1.00**				
	Sulfatase	-0.41	-0.08	0.28	0.26	0.50	-0.50	-0.30	-0.15	-0.44	-0.04	0.20	0.04	0.33	-0.48	-0.46	-0.46	-0.08	-0.46	-0.37	-0.53	-0.13	-0.06	-0.26	-0.15	-0.11	0.38	-0.31	-0.34	0.46	0.28	0.48	-0.25	**1.00**			
	Desidrogensae	-0.06	-0.16	-0.02	0.18	0.14	-0.26	0.17	0.10	-0.35	-0.21	0.25	0.58	0.05	0.00	-0.39	-0.36	0.43	0.09	-0.42	-0.20	-0.47	0.61	-0.35	-0.17	-0.37	0.06	-0.42	-0.36	0.06	0.48	0.29	0.13	-0.15	**1.00**		
Physical	**Moisture**	-0.04	0.25	0.21	0.08	-0.13	-0.19	0.15	0.63	-0.23	-0.13	0.26	0.04	0.10	-0.15	-0.25	0.07	0.03	0.01	-0.19	0.04	-0.20	-0.09	-0.02	-0.14	0.00	0.21	0.08	-0.23	0.02	0.30	-0.23	0.42	-0.26	0.21	**1.00**	
	Coarse Elements	0.72	0.62	0.01	0.19	-0.31	0.43	0.71	0.34	0.37	-0.14	0.25	0.31	0.03	0.67	0.52	0.59	-0.44	0.57	0.08	0.13	0.02	0.43	0.41	0.33	0.11	0.14	-0.05	0.19	-0.61	-0.34	-0.17	-0.18	-0.26	0.13	-0.14	**1.00**

Annex 4- Bivariate Pearson correlations table for all factors analysed, between plots in different regions.

P1 VS P2		Physicochemical				Available Nutrients									Total Nutrients									Exchange Cations						Enzymatic Activities						Physical Prop.	
		pH	*CE*	*SOC*	*N*	*Fe*	*Mn*	*Zn*	*Cu*	*Mg*	*Na*	*K*	*P*	*K*	*Fe*	*Mn*	*Zn*	*Cu*	*Ca*	*Mg*	*Na*	*K*	*P*	*Ca*	*Mg*	*Na*	*K*	*Al*	*CTC*	Phosphatase	β-glucosidase	Cellulase	Urease	Sulfatase	Dehydrogenase	Moisture	Coarse elements
Physicochemical	*pH*	**1.00**																																			
	CE	0.60	**1.00**																																		
	SOC	-0.16	0.53	**1.00**																																	
	N	-0.28	0.49	0.75	**1.00**																																
Available Nutrients	Fe	-0.67	-0.17	0.17	0.56	**1.00**																															
	Mn	0.71	0.26	-0.41	-0.42	-0.39	**1.00**																														
	Zn	0.59	0.73	0.17	0.36	-0.05	0.53	**1.00**																													
	Cu	0.42	0.69	0.19	0.26	-0.04	0.09	0.62	**1.00**																												
	Mg	0.80	0.24	-0.50	-0.58	-0.56	**0.89**	0.34	0.11	**1.00**																											
	Na	-0.02	-0.23	-0.42	-0.27	-0.10	-0.09	-0.39	-0.09	0.23	**1.00**																										
	K	-0.24	0.33	0.64	0.69	0.42	-0.37	0.31	0.29	-0.63	-0.55	**1.00**																									
	P	-0.07	0.03	0.03	0.38	0.17	-0.32	-0.01	-0.05	-0.33	0.19	0.18	**1.00**																								
	K	-0.47	0.03	0.48	0.58	0.35	-0.58	-0.18	-0.10	-0.76	-0.34	0.70	0.42	**1.00**																							
Total Nutrients	Fe	0.63	0.62	0.15	0.21	-0.21	0.61	0.80	0.30	0.53	-0.14	0.10	-0.15	-0.41	**1.00**																						
	Mn	0.84	0.42	-0.34	-0.38	-0.56	**0.90**	0.50	0.16	**0.95**	0.05	-0.50	-0.31	-0.61	0.64	**1.00**																					
	Zn	0.82	0.79	0.12	0.04	-0.50	0.70	0.67	0.47	0.71	-0.01	-0.15	-0.17	-0.36	0.74	0.84	**1.00**																				
	Cu	-0.50	-0.62	0.00	-0.05	0.01	-0.54	-0.42	-0.30	-0.50	0.11	0.00	0.04	0.11	-0.34	-0.56	-0.60	**1.00**																			
	Ca	**0.90**	0.55	-0.18	-0.20	-0.55	0.62	0.50	0.39	0.76	0.16	-0.40	0.13	-0.58	0.59	0.76	0.77	-0.47	**1.00**																		
	Mg	0.66	0.09	-0.47	-0.67	-0.63	0.74	0.06	-0.02	**0.92**	0.34	-0.82	-0.38	-0.77	0.28	0.84	0.60	-0.33	0.68	**1.00**																	
	Na	0.69	0.07	-0.48	-0.76	-0.74	0.65	0.01	0.04	0.84	0.26	-0.67	-0.29	-0.73	0.26	0.72	0.51	-0.31	0.71	**0.88**	**1.00**																
	K	0.61	0.13	-0.36	-0.60	-0.61	0.51	-0.05	0.05	0.80	0.45	-0.81	-0.35	-0.70	0.14	0.71	0.53	-0.27	0.62	**0.94**	0.77	**1.00**															
	P	0.17	0.05	-0.16	0.25	0.09	-0.02	0.21	0.02	-0.04	0.23	-0.02	**0.91**	0.11	0.06	-0.03	0.00	0.02	0.36	-0.12	-0.13	-0.12	**1.00**														
Exchange	Ca	**0.87**	0.48	-0.30	-0.44	-0.62	0.66	0.37	0.46	**0.86**	0.23	-0.51	-0.32	-0.63	0.41	0.84	0.77	-0.49	0.80	0.82	0.74	**0.85**	-0.06	**1.00**													
	Mg	0.77	0.21	-0.53	-0.60	-0.50	0.66	0.32	0.32	0.84	0.14	-0.59	-0.34	-0.73	0.29	0.77	0.51	-0.39	0.70	0.79	0.71	0.79	-0.03	**0.91**	**1.00**												
	Na	0.69	0.26	-0.39	-0.59	-0.55	0.53	0.06	0.28	0.80	0.35	-0.70	-0.30	-0.68	0.12	0.70	0.55	-0.47	0.71	**0.87**	0.81	**0.93**	-0.10	**0.91**	**0.88**	**1.00**											

	K	-0.41	0.27	0.68	0.72	0.43	-0.52	0.13	0.14	-0.74	-0.50	**0.93**	0.18	0.83	-0.09	-0.57	-0.24	0.00	-0.56	**-0.86**	-0.77	-0.80	-0.09	-0.59	-0.68	-0.72	**1.00**										
	Al	-0.15	0.03	0.09	0.08	-0.08	-0.03	-0.15	-0.08	-0.15	-0.28	0.16	-0.20	0.50	-0.13	0.02	0.09	-0.05	-0.28	-0.11	-0.09	-0.19	-0.40	-0.14	-0.30	-0.21	0.29	**1.00**									
	CTC	0.57	0.05	-0.58	-0.56	-0.45	0.59	0.03	0.03	0.85	0.72	-0.75	-0.13	-0.73	0.30	0.71	0.51	-0.30	0.64	0.85	0.75	0.82	0.10	0.75	0.68	0.77	-0.80	-0.26	**1.00**								
Enzymatic Activities	**Fosfatases Ácidas**	**-0.87**	-0.27	0.50	0.57	0.64	-0.74	-0.41	-0.32	-0.79	0.02	0.27	0.16	0.43	-0.39	-0.76	-0.58	0.41	-0.69	-0.62	-0.72	-0.52	-0.06	-0.79	-0.80	-0.63	0.44	0.02	-0.56	**1.00**							
	Glucosidases	-0.76	-0.22	0.40	0.59	0.61	-0.64	-0.07	-0.12	-0.84	-0.25	0.51	0.36	0.48	-0.28	-0.79	-0.59	0.40	-0.62	-0.81	-0.76	-0.80	0.18	**-0.86**	-0.78	-0.80	0.58	-0.09	-0.74	0.80	**1.00**						
	Celulases	-0.40	-0.17	0.20	0.28	0.68	-0.38	-0.13	-0.07	-0.42	-0.15	0.48	0.00	0.14	-0.06	-0.52	-0.53	0.08	-0.37	-0.54	-0.42	-0.49	-0.09	-0.46	-0.30	-0.40	0.36	-0.32	-0.39	0.35	0.33	**1.00**					
	Ureases	-0.48	-0.03	0.28	0.49	0.27	-0.41	-0.10	0.05	-0.59	-0.09	0.38	0.40	0.67	-0.27	-0.47	-0.17	0.28	-0.39	-0.53	-0.49	-0.56	0.17	-0.55	-0.73	-0.59	0.45	0.59	-0.47	0.46	0.53	-0.22	**1.00**				
	Sulfatase	-0.41	-0.08	0.28	0.26	0.50	-0.50	-0.30	-0.15	-0.44	-0.04	0.20	0.04	0.33	-0.48	-0.46	-0.46	-0.08	-0.46	-0.37	-0.53	-0.13	-0.06	-0.26	-0.15	-0.11	0.38	-0.31	-0.34	0.46	0.28	0.48	-0.25	**1.00**			
	Desidrogensae	-0.06	-0.16	-0.02	0.18	0.14	-0.26	0.17	0.10	-0.35	-0.21	0.25	0.58	0.05	0.00	-0.39	-0.36	0.43	0.09	-0.42	-0.20	-0.47	0.61	-0.35	-0.17	-0.37	0.06	-0.42	-0.36	0.06	0.48	0.29	0.13	-0.15	**1.00**		
Physical	**Moisture**	-0.04	0.25	0.21	0.08	-0.13	-0.19	0.15	0.63	-0.23	-0.13	0.26	0.04	0.10	-0.15	-0.25	0.07	0.03	0.01	-0.19	0.04	-0.20	-0.09	-0.02	-0.14	0.00	0.21	0.08	-0.23	0.02	0.30	-0.23	0.42	-0.26	0.21	**1.00**	
	Coarse Elements	0.72	0.62	0.01	0.19	-0.31	0.43	0.71	0.34	0.37	-0.14	0.25	0.31	0.03	0.67	0.52	0.59	-0.44	0.57	0.08	0.13	0.02	0.43	0.41	0.33	0.11	0.14	-0.05	0.19	-0.61	-0.34	-0.17	-0.18	-0.26	0.13	-0.14	**1.00**

Annex 5- Field Management Notebook from Quinta dos Murças (P1 and P2)

Date	Product	Active substance	Product/ha (lt or kg)	Phenologic growth state	Baggiolini Scale	Plot
02/04/2020	Copper Nordox	Copper	1.0	Inflorescence emergence	F/G	P1
02/04/2020	Kumulus	Sulfer	0.1	Inflorescence emergence	F/G	P2
	Copper Nordox	Copper	1.0	Inflorescence emergence	F/G	
	Kumulus	Sulfer	1.3	Inflorescence emergence	F/G	
18/04/2020	Copper Nordox	Copper	0.8	Inflorescence emergence	G/H	P2
	Kumulus	Sulfer	1.1	Inflorescence emergence	G/H	
	Copper Nordox	Copper	1.0	Inflorescence emergence	G/H	
	Kumulus	Sulfer	1.8	Inflorescence emergence	G/H	
	Copper Nordox	Copper	0.8	Inflorescence emergence	G/H	
25/04/2020	Kumulus	Sulfer	1.1	Inflorescence emergence	H	P2
	Copper Nordox	Copper	1.0	Inflorescence emergence	H	
	Kumulus	Sulfer	1.8	Inflorescence emergence	H	
	Copper Nordox	Copper	0.6	Inflorescence emergence	H	
	Kumulus	Sulfer	1.1	Inflorescence emergence	H	
	Copper Nordox	Copper	1.0	Inflorescence emergence	H	
14/05/2020	Kumulus	Sulfer	2.1	Flowering	I	P1
	Copper Nordox	Copper	1.6	Flowering	I	
	Kumulus	Sulfer	4.0	Flowering	I	
	Copper Nordox	Copper	1.3	Flowering	I	
	Kumulus	Sulfer	3.1	Flowering	I	
28/05/2020	Copper Nordox	Copper	2.4	Fruit set	I/J	P2
	Kumulus	Sulfer	6.0	Fruit set	I/J	
	Copper Nordox	Copper	1.3	Fruit set	I/J	
	Kumulus	Sulfer	10.4	Fruit set	I/J	
06/06/2020	Copper Nordox	Copper	1.6	Development of Fruits	K	P2
	Kumulus	Sulfur	13.3	Development of Fruits	K	
	Bago D'Ouro	Sulfur	10.0	Development of Fruits	K	
	Bago D'Ouro	Sulfur	10.0	Development of Fruits	K	
	Bago D'Ouro	Sulfur	10.0	Development of Fruits	K	
17/06/2020	Bago D'Ouro	Sulfur	10.6	Development of Fruits	L	P2
	Align	Natural insecticide	0.6	Development of Fruits	L	

Annex 6- Field Management Notebook from Quinta do Monte D'Oiro (P3)

Date	Product	Active Substance	Product/ha (lt or kg)	Phenologic growth state	Baggiolini Scale
01/02/2021	Klik Extra	Paraffinic Oil	15	Sprouting	A
11-03-2021 to 23/3/2021	Kumullus	Sulfur	10	Sprouting	B/C/D
	Novicure	Copper	0.5	Sprouting	
	Retenol	Pine Oil	0.6	Sprouting	
02/04/2021	Stulln	Sulfur	6	Inflorescence emergence	F/G
	Kados	Copper	1	Inflorescence emergence	
	Myr Fe	Amino acids + Fe	0.5	Inflorescence emergence	
	Myr N	Amino acids +N	0.5	Inflorescence emergence	
	Myr Mix	Amino acids + Micronutrients	1	Inflorescence emergence	
08-04-2021 and 9-4-2021	Stulln	Sulfur	6	Inflorescence emergence	F/G
	Novicure	Copper	0.8	Inflorescence emergence	
	Sergomil	Copper	1	Inflorescence emergence	
	Myr Mix	Amino acids + Micronutrients	1	Inflorescence emergence	
	Myr N	Amino acids +N	0.5	Inflorescence emergence	
	Myr B	Amino acids + B	0.5	Inflorescence emergence	
19/4/2021 and 20/4/2021	Nordox 75	Copper	0.8	Inflorescence emergence	G/H
	Stulln	Sulfur	8	Inflorescence emergence	
	Myr Ca	Amino acids + Ca	0.5	Inflorescence emergence	
	Myr B	Amino acids + Br	0.5	Inflorescence emergence	
	Retenol	Pine Oil	0.5	Inflorescence emergence	
23/04/2021	Armicarb	Potassium (P)	5	Inflorescence emergence	H
	Vitisan	Potassium (P)	4	Inflorescence emergence	
29/04/2021	Kocide 2000	Copper	1	Inflorescence emergence	H
	Myr N	Amino acids + N	1	Inflorescence emergence	
	Myr Fe	Amino acids + Fe	0.5	Inflorescence emergence	
	Myr B	Amino acids+ B	0.5	Inflorescence emergence	
	Retenol	Pine Oil	0.5	Inflorescence emergence	
07/05/2021	Stulln	Sulfur	8	Flowering	H/I
	Novicure	Copper	1.3	Flowering	
	Myr N	Amino acids + N	1	Flowering	
	Myr B	Amino acids + B	1	Flowering	
18/05/2021	Sulfur diamante	Sulfur	30	Flowering	I
	Clay	Bentonite Clay	30	Flowering	

21/05/2021	Kocide 2000	Copper	1	Fruit set	I/J
	Myr Mix	Amino acids+ Micronutrients	1	Fruit set	
				Fruit set	
31/05/2021	Kocide 2000	Copper	1	Fruit set	J
	Stulln	Sulfer	8	Fruit set	
	Myr Mg	Amino acids+ Mg	1.5	Fruit set	